EFFECT OF CONCENTRATION, EXPOSURE TIME, TEMPERATURE, AND RELATIVE HUMIDITY ON THE TOXICITY OF SULFUR DIOXIDE TO THE SPORES OF BOTRYTIS CINEREA

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SUMMARY

When spores of *Botrytis cinerea* are exposed to SO_2 gas, the subsequent reduction in spore germination is quantitatively proportional to the SO_2 concentration and the exposure time. The toxicity of SO_2 increases with increasing relative humidity. In an atmosphere of 96% RH, SO_2 is more than 20 times as effective as at 75% RH. The toxicity also increases about 1.5 times for each 10°C rise in temperature between 0° and 30°C.

INTRODUCTION.—SO_2 fumigation to control decay of vinifera table grapes caused by *Botrytis cinerea* Fr. is an established commercial practice in California. The grapes are usually treated immediately after harvest and at intervals during storage (8). The initial SO_2 treatment may be applied in special gassing rooms, precooling rooms, storage rooms, trucks, or railroad cars. When properly done, this treatment kills fungi on or near the surface of the fruit, but does not eradicate disease organisms that have become established in the fruit (7). The initial fumigation is sometimes applied under uncontrolled conditions that contribute to irregular control of decay and to injury of the fruit. Subsequent fumigations of the grapes in storage restrict the spread of infections remaining after the initial treatment.

Although the effect of SO_2 fumigation on subsequent decay of grapes has been studied quite extensively (8), no attempt has been made to determine the effect of SO_2 on the spores and mycelium of any pathogen of grapes under controlled conditions. Therefore, we studied the effect of concentration, exposure time, temperature, and relative humidity on the toxicity of SO_2 gas to the spores of *B. cinerea*.

METHODS.—The culture of *B. cinerea* used in these experiments was isolated from an infected Emperor grape and was maintained on potato-dextrose agar by mass spore transfer. Cultures were grown at 20°C on 7 ml of medium in a 50-ml jar plugged with cotton. Under these conditions, the fungus grew rapidly and sporulated uniformly, producing mature spores by the tenth day after inoculation. Cultures were started at about 10-day intervals to provide a constant supply of vigorous spores. The spores did not vary significantly in germination or in response to SO_2 until the cultures were more than 30 days old. Cultures older than this were not used.

Before fumigation, the spores were dusted lightly on a patch of lens paper or 1.5-mil. polyethylene film about 2 cm square. Lens paper was used for fumigation of wet spores and polyethylene for dry spores. The paper and spores were placed momentarily on moist filter paper and were moistened by capillary action. The paper or polyethylene patches were usually placed on a microscope slide during fumigation. The water held the paper on the slide and the polyethylene was held by a small amount of lanolin or stopcock grease.

The fumigation chamber was 2.7 cm in diameter and 18 cm long, having a volume of about 100 ml. A continuous stream of SO_2 gas entered the chamber at the bottom and flowed upward around the spores. The patches holding the spores could be removed at intervals without interrupting the treatment. Since it was impossible to humidify SO_2 gas mixtures directly, the desired concentrations of SO_2 and water vapor were obtained by mixing streams of SO_2 and humidified air. The air was humidified to 97-100% RH before mixing by passage through a series of 3 water bubblers immersed with the fumigation chambers in a stirred, thermostatically controlled water bath. Stock concentrations of SO_2 (8,500, 5,200, 2,500, and 900 ppm) were prepared by diluting pure SO_2 with N_2. The strength of these mixtures was checked by iodine titration (1). By appropriate dilution, many concentrations of SO_2 at various levels of relative humidity were maintained. Flow rates were controlled with 2-stage pressure regulators, needle valves, and rotameter-type flowmeters. The relative humidity levels and SO_2 concentrations given in the text are based on dilution, but also were checked periodically by measuring the relative humidity with thermocouples, used as wet and dry bulbs, and by measuring the SO_2 concentration with a Liston-Becker infrared gas analyzer. The flow rate through the fumigation chamber was 1 liter/minute for all tests except the temperature experiments, in which the rate was 600 ml/minute. No difference in SO_2 toxicity was observed due to change in flow rate.

After fumigation, the patches were inverted momentarily on the surface of cold gelatin in petri plates. The spores adhered to the gelatin in the same relative positions occupied during treatment. No nutrient was added to the gelatin, and the pH was about 6.

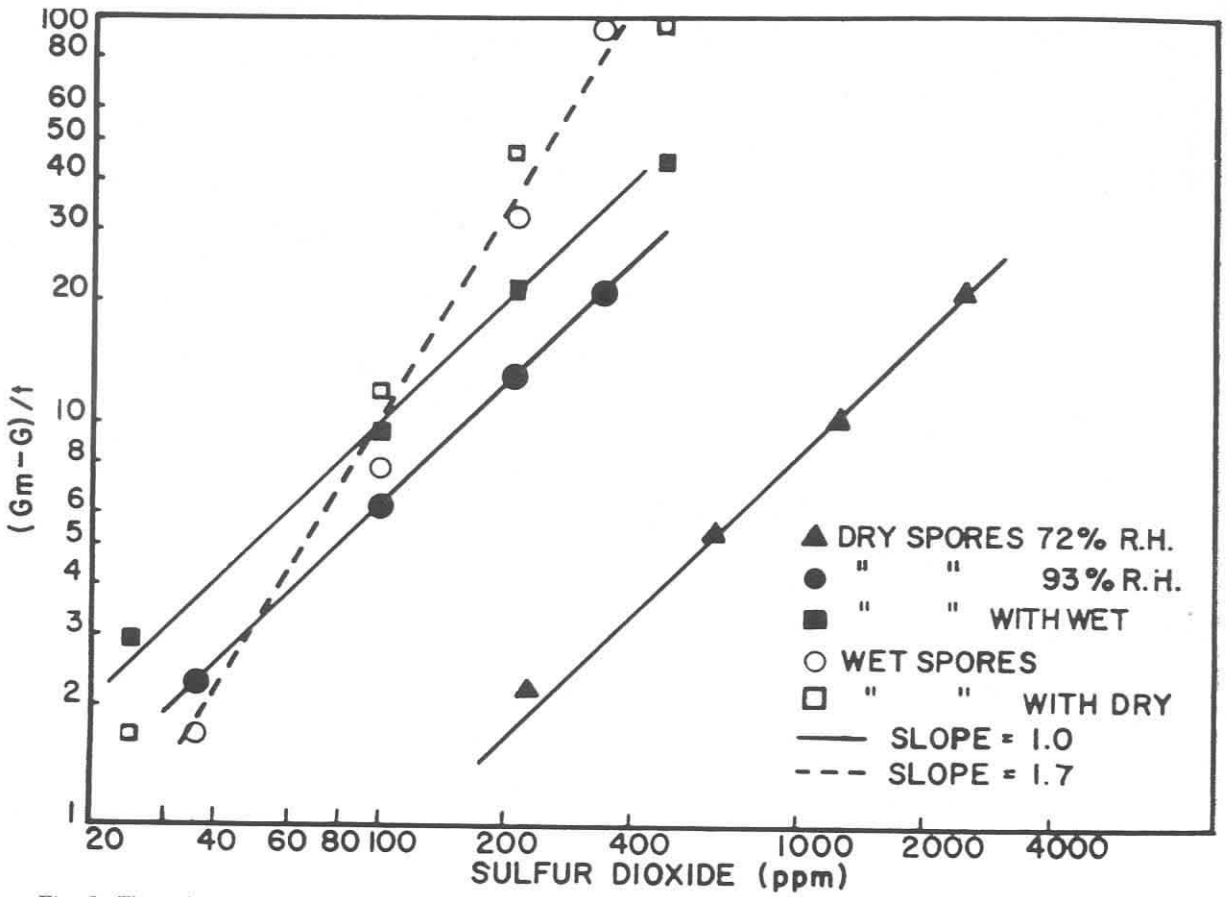

Fig. 1. The reduction in percentage of spore germination/minute exposure to SO_2 as a function of the SO_2 concentration on logarithmic coordinates at 20°C. Wet paper in the fumigation chamber increased relative humidity to nearly 100%.

The spores were incubated at 20°C for about 20 hours. Then, total spores and germinated spores were counted in 5 microscope fields (× 430), each containing 20-25 spores, and percentage of germination was calculated.

Each experiment was replicated 3-6 times, depending on the variability of the results. Data were analyzed statistically. Probability levels exceeding 99% were considered significant.

The following equation relating spore germination to SO_2 concentration and exposure time was used to condense and summarize the data: equation 1, $Go-G = k\,C^n t$, where Go = percentage of germination of untreated spores, G = percentage of germination of treated spores, C = concentration of SO_2, t = exposure time, and k = a proportionality constant.

The equation may be rearranged and logarithms of each side taken as follows: equation 2, $\log Go-G/t = \log k + n \log C$. The value of n was determined from the slope formed by plotting $\log Go-G/t$ *vs.* $\log C$ (Fig. 1.) After determining n, k was calculated directly. This equation simply states that the reduction in germination is proportional to the effective dose ($C^n t$) of SO_2. At any given concentration of SO_2, equation 1 is formally identical with the lower terms of a polynomial and the linearity of the response to exposure time was tested statistically using the polynomial method (5). The calculated response and the experimental points also may be compared (Fig. 2).

RESULTS.—*SO_2 concentration and exposure time.*— The toxicity of SO_2 depended on the concentration and the exposure time. When exposure time and environmental conditions were constant, germination was a function of SO_2 concentration. When the spores were dry during treatment, germination was a linear function of SO_2 concentration and the value of n was 1.0. If spores were wet, germination became a power function of the concentration and the value of n was 1.7 (Fig. 1. 2). This effect of water on the value of n cannot be attributed to a change in concentration of SO_2 due to absorption by the wet paper. When wet spores on wet paper and dry spores on polyethylene were placed side by side on a microscope slide, and exposed to SO_2 gas, the respective values of n were almost identical to those obtained in the previous separate experiments (Fig. 1). Therefore, this change in n must be due to the water immediately surrounding the spore or to the effect of water on the physiology of the spore.

The graphs (Fig. 2, 3) and the statistical treat-

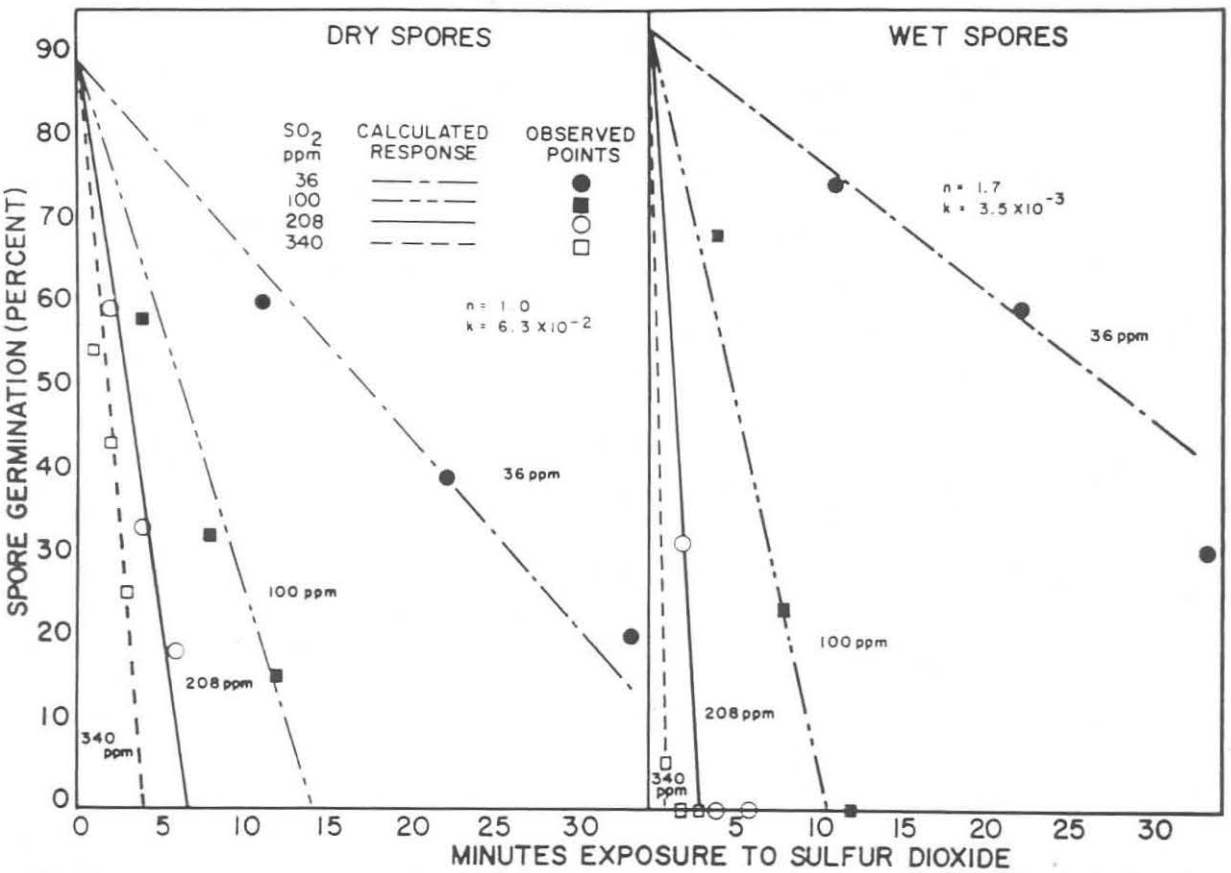

Fig. 2. Spore germination as a function of SO_2 concentration and exposure time at 20°C and 96% relative humidity. Dry spore treatment means less than 69% and wet spore treatment means less than 72% are significantly different from the checks (90% and 94%, respectively).

ment showed that germination decreased linearly with increased time of exposure to SO_2.

Relative humidity and temperature.—Relative humidity had a great influence on the toxicity of SO_2 to dry spores. A concentration of 100 ppm SO_2 for 12 minutes was very effective at 96% RH, but had no significant effect on germination at 75% RH. Since a wide range of relative humidity could not be studied effectively at any single dose of SO_2, the desired comparisons were based on values of k assuming that $n = 1$ for dry spores at any relative humidity (Fig. 1). The toxicity of SO_2 at 96% RH was 23 times as great as at 75% RH and at least 11,000 times as great as at 0% RH (Table 1). The k values for wet and dry spores cannot be compared directly because of differences in the exponent n. Based on the value of k, the toxicity of SO_2 also increased about 1.5 times for each 10° rise in temperature from 1° to 30°C (Fig. 3).

Since relative humidity at any given vapor pressure is dependent on temperature, the combined effect of temperature and relative humidity must be considered. The data clearly show that k is not a function of vapor pressure (Fig. 4). When the vapor pressure was lowered by reducing the temperature at a constant relative humidity, much less effect on k was observed than by reducing the relative humidity at a constant temperature.

TABLE 1. Germination of *Botrytis cinerea* spores after exposure to different concentrations of SO_2 gas while dry at indicated relative humidities at 20°C: means less than 69% are significantly different from the checks

Relative humidity (%)	SO_2 (ppm)	% germination after exposure for indicated time (min.)[a] 0	4	8	12	16	24	60	k[c]
–	0	93							
93 – 96	100		66	39	24				6.6×10^{-2}
87 – 90	520		60	20	1				1.6×10^{-2}
73 – 75	2100		64	41	30				2.8×10^{-3}
58 – 60	3400		72	69	55				9.3×10^{-4}
39 – 40	5100		89	81	69				3.8×10^{-4}
19 – 20	6800			87		88[b]	91[b]		3.1×10^{-5}
0	8500			91		92[b]	88	90	5.9×10^{-6}

[a] From 5 replications, 2 check plates/replicate.
[b] One missing value.
[c] $k = \frac{Go - G}{C^n t}$, $n = 1$. See text.

DISCUSSION.—These data provide some indication of the mechanism of the action of SO_2 on *Botrytis* spores. The effect of relative humidity on SO_2 toxicity indicates that the hydrated form of SO_2 (sulfurous acid) is the active agent. Sulfurous acid is a more effective fungicide than its salts (3). Since sulfurous acid is

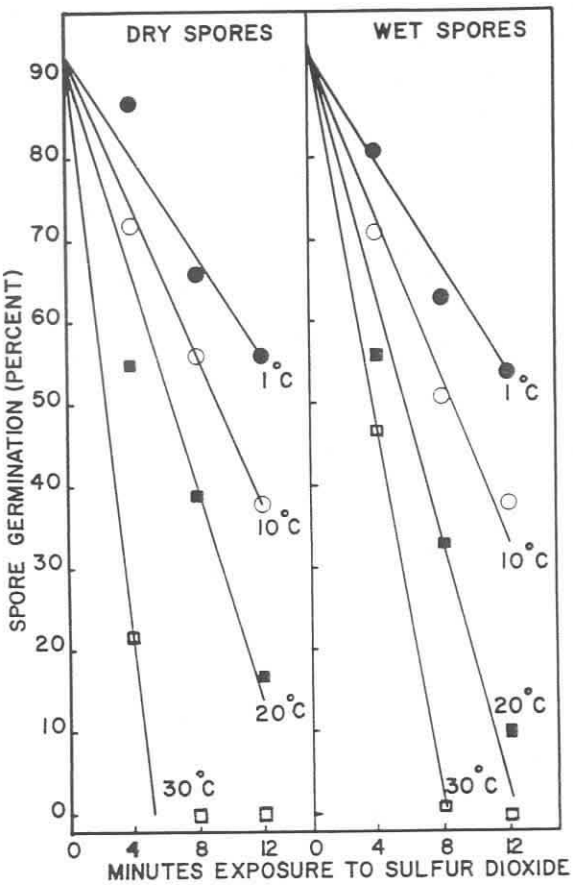

Fig. 3. Spore germination as a function of temperature and time of exposure to 100 ppm SO_2 at 96% relative humidity. Treatment means less than 81% are significantly different from the checks.

highly reactive, it may inhibit spore germination by combining directly with essential cell constituents. The failure of the spores to recover from SO_2 inhibition after they are removed from the SO_2 atmosphere may be attributed to an essentially irreversible combination of the sulfurous acid with the cellular material. The fact that SO_2 became more toxic as the temperature was increased and that spore germination was reduced linearly as the exposure time was increased supports this view (4). The small effect of temperature suggests that physical processes such as the hydration of SO_2 or the diffusion of the sulfurous acid to sensitive sites in the cell limit the rate of reaction (6).

Cant and Nelson (2) suggested that decay control by SO_2 is a function of the SO_2 "concentration-time factor" (the dose or Ct). If $n = 1$ for this process, good correlation between dose and effect may be expected. If $n \neq 1$, the more general quantity ($c^n t$) or "effective dose" should be substituted and may prove equally useful. If the value of n is not known from experiment, dosage calculations should be used with caution.

In these experiments, decay control was not considered. If the control of gray mold of grapes is a function of the ability of SO_2 to inhibit spore germination, however, the temperature and relative humidity during treatment may greatly influence the effectiveness of fumigation. A small decrease in temperature may increase the relative humidity and the net effectiveness of the treatment, or warm fruit in cool air may lower the relative humidity surrounding the fruit and decrease the effect of the SO_2 treatment. Data correlating spore germination and infectivity under various controlled conditions are needed. Additional observations of temperature and relative humidity under commercial conditions may indicate whether fumigation practices could be improved by better control of environmental conditions.

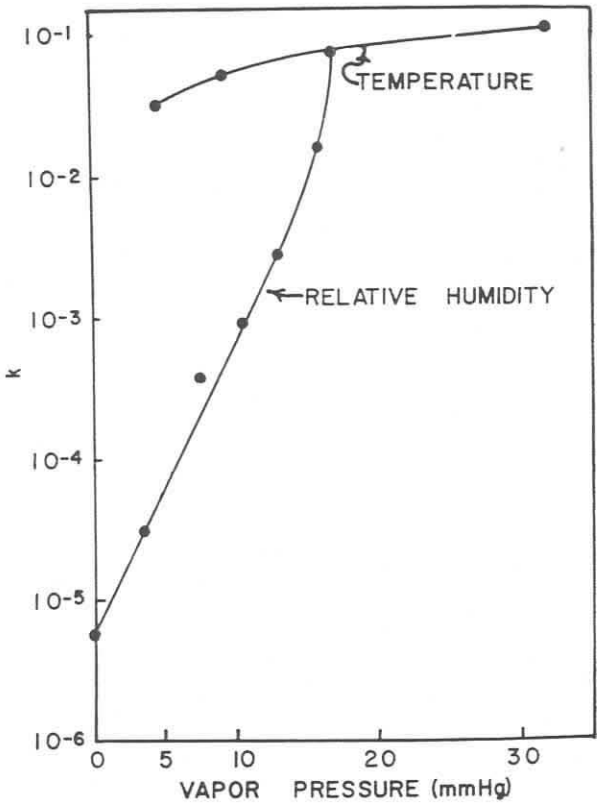

Fig. 4. The value of k as a function of vapor pressure. The vapor pressure was varied by a change in temperature from 1° to 30°C at constant relative humidity or by changing the relative humidity from 0% to 96% at constant temperature. The data are from Fig. 3 and Table 1. Logarithmic scale was used only for convenience.

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